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Clin. Orth. Relat. Res. 164 1982 pages 265-70 Biomed.  
Res. 2(5) 1981 pages 466-471.

(58) Field of search

C3H

(54) Artificial bone forming composition

(57) An artificial bone-forming biomaterial comprising a mixture of a bone-forming factor and a first carrier selected from collagen, its derivatives and denatured substances and a method of making the biomaterial.

GB 2 164 042 A

# POOR QUALITY

1

GB 2 164 042 A

1

## SPECIFICATION

### Artificial bone-forming biomaterial and method of manufacturing the same

5 This invention relates to a biomaterial including a bone-forming factor used in a surgical field such as orthopedic surgery and oral surgery and to a method of manufacturing the biomaterial. 5

Previously, when a piece of bone in a living body was replaced with an artificial substitute, it has been general practice to excise a section of the bone from another area of the same living body and to use that section as a transplant. Such autoplasmic transplantation is desirable in that it has excellent biocompatibility with the bone it replaces. 10

It will be self-evident that there is a limit to the amount of bone which is available for autoplasmic transplantation. The amount of surgery required to collect bone increases and the pain experienced by the patient is great. For that reason, when a deficiency in the bone extends over a large area, an artificial biomaterial having affinity with the living body such as metal or ceramics has been used for filling the deficiency and for fixation of the artificial material to the bone. 15

However, the use of a wholly artificial biomaterial has the disadvantage that rapid adhesion of the bone to the biomaterial with sufficient strength is difficult. What is considered to be the main cause of this difficulty is that bone tissue is not formed in sufficient amounts. Accordingly, it is difficult to obtain rapidly strong and hard tissue of a living body by the surgical treatment in which a conventional artificial biomaterial is used. 20

An object of this invention is to obviate or mitigate the drawbacks inherent in the conventional artificial biomaterials described above. 20

According to this invention there is provided an artificial bone-forming biomaterial comprising: a mixture of bone-forming factor and a first carrier for said factor, said carrier being selected from collagen and derivatives thereof and denatured substances. 25

More preferably the invention provides an artificial bone-forming biomaterial comprising: a mixture of bone-forming factor and a first carrier for said factor, said carrier being selected from collagen, derivatives thereof and denatured substances, and a second carrier for said mixture, said carrier being a formed body of metallic or synthetic resinous material wherein said mixture is attached to or impregnated into and carried by said second carrier. 30

Preferably also the bone-forming factor is a bone-forming factor extracted from Dunn osteosarcoma or a human bone-forming factor extracted from a human osteosarcoma. 30

The factor is carried stably by the first carrier in the living body to permit the formation of bone by the factor and to make the collagen-based first carrier itself eventually be absorbed into the body. In a further developed aspect of the invention, a second carrier, in addition to the first carrier, is used for mechanical reinforcement. When the biomaterial of the invention is used for compensating for and fixing broken or otherwise affected bones, it promotes quick growth and restoration of bone to enable restoration of a bone tissue with sufficient strength. 35

The essential element of the artificial biomaterial in the invention is a bone-forming factor, and this factor is a substance supposed for long to exist in a living body. 40

The function of the bone-forming factor is to act extracellularly on undifferentiated mesenchymal cells and to induce the phenotype of the cells to chondrocytes and osteoblasts to thereby form a bone tissue locally. The applicant, after years' researches, developed a method of separating a bone-forming factor (mouse bone-forming factor) from Dunn osteosarcoma and refining the factor and already reported the substance in "BIOMEDICAL RESEARCH" 2 (5) 466-471 in 1981. The substance is a basic and hydrophobic polypeptide of about 20,000 by molecular weight. Further, the applicant has recently separated and refined a similar bioactive bone-forming factor also from a human osteosarcoma transplanted in the animals by way of generation-to-generation transplantation. 45

The human bone-forming factor also has substantially the same biochemical properties as the bone-forming factor obtainable from the above osteosarcoma, and accordingly this human bone-forming factor was used in the embodiments of the invention from the viewpoint of antigenicity. 50

In this manner, the human bone-forming factor develops a bone-forming action in the living body, but a certain type of carrier (or bed) is required in order for the factor to lead to bone formation in a specified region of the living body, and the amount of bone formed is regulated by the amount of carrier including and carrying the bone-forming factor. Accordingly, in order to carry the bone-forming factor in the living body, the following first and second carriers are provided, respectively. 55

The first carrier contains and carries the bone-forming factor and makes it possible to form a bone, while the second carrier is a formed body retaining the first carrier thereon or therein, having a necessary shape together with necessary strength. 60

The first carrier is required to have the properties 60

1) that when embedded into a living body, the carrier should not induce a foreign matter reaction;

2) that the carrier should have affinity with bone;

3) that the carrier can always procure an industrially constant, ready and low-price supply;

4) that the carrier should be such as to be stably retainable without spoiling the properties of the bone-forming factor and to be miscible in any desired ratio; 65

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- 5) that the carrier should be ultimately absorbable into the living body; and  
6) that the carrier should be readily attachable to the second carrier.

The second carrier is required to have the properties

- 1) that the carrier should have the properties of the first carrier specified by items 1 to 4;  
2) that the properties of the carrier should remain the same over a long period of time;  
3) that the carrier should be easy to make physical bond with the first carrier; and  
4) that the carrier should be high in mechanical strength.

Various researches made on the material for obtaining the first carrier having such properties resulted in the finding that collagen, its derivatives and denatured substances could meet the required properties.

- Incidentally, it is well known that collagen (inclusive of its derivatives and denatured substances) based on the first carrier is protein generally low in antigenicity, and it is also known that the main part which constitutes the cause of antigenicity of collagen lies in the telopeptide part which forms a molecular distal end part. Accordingly, for example, solubilized collagen containing substantially no telopeptide solubilized and refined by subjecting cowhide to well-known enzymatic treatment and alkaline treatment is preferred for the object of the invention, but the first carrier is not limited to solubilized collagen. It will readily be appreciated that gelatin (inclusive of decomposition products and derivatives) which is the denatured substance of collagen is also applicable to the construction of the invention.

- The requisites for the second carrier are such as described above, and out of the requisites, ceramic materials, special metals and synthetic resins are known to be excellent as a material producing no foreign matter reaction with the living body and which has affinity with the living body and is high in mechanical strength. Out of the materials mentioned above, ceramic materials excellent in affinity with the living body are listed in Table I.

- In the first manner of the invention (corresponding to embodiments 1 and 3 to be later described), the example wherein the bone-forming factor is mixed with the first carrier is shown, while in the second manner of the invention (corresponding to embodiments 2 and 4 to be later described), the example wherein the second carrier is impregnated with and carried by the above mixture is shown.

# POOR QUALITY

3

GB 2 164 042 A

3

TABLE I

	Type of ceramics	Physical properties	Bending strength (Kg/cm <sup>2</sup> )	Hardness	Bulk specific gravity	Resistance to chemicals 95% H <sub>2</sub> SO <sub>4</sub> in boiling liquid mg/cm <sup>2</sup> /day	
5							5
10	Alumina ceramic (Al <sub>2</sub> O <sub>3</sub> )		3,200	1,000 - 2,300 (H V)	3.6 - 4.0	0.1	10
15	Sapphire (Al <sub>2</sub> O <sub>3</sub> )		7,000	2,300 (H V)	3.97	0.1	15
	Zirconia (ZrO <sub>2</sub> )		10,000	1,250 (H V)	5.9	0.8	
20	Silicon carbide (SiC)		5,000	94 (HRA)	2.2 - 3.1	0.04	20
25	Silicon nitride (Si <sub>3</sub> N <sub>4</sub> )		5,000	87 - 91 (HRA)	2.9 - 3.3	0.42	25
30	Calcium phosphate Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>		1,400	—	3.0 - 3.05	Soluble in acid, hard to dissolve in base	30
35	Hydroxylapatite Ca <sub>10</sub> (PO <sub>4</sub> ) <sub>6</sub> (OH) <sub>2</sub>		600 - 2,000	400 - 600	3.06 - 3.13	Same as above	35
	Glass ceramic (containing apatite)		1,500 - 1,700	600 - 700	About 3.5	Same as above	

Of the ceramic materials shown in Table 1, alumina ceramics is the most general material and has no harmful effect on the living body and is high in affinity therewith. The materials having affinity and high in mechanical strength are sapphire and zirconia ceramics. Other materials such as calcium phosphate and hydroxylapatite which are substantially akin to living body bone are somewhat low in mechanical strength, but they are characterized by their excellency in assimilability and adhesibility to the living body bone. Accordingly, the optimum ones of the ceramic materials mentioned above are used depending upon where they are used and upon the purposes they are used for. As for the properties of the ceramic materials, those which are fit for their respective purposes are such as ones of dense type or porous type may be used. The minimum required amount of the bone-forming factor in the biomaterial of the invention differs depending upon a degree of refinement of the bone-forming actor, and in the factors obtained by the method according to the literature described in the specification, bone formation is possible by collagen:factor < 100: 0.5 (ratio by weight), but in order to increase and assure the effect of bone formation, it is desirable to place a ratio of collagen to bone-forming factor in the range of 100:1 - 10 (ratio by weight), but the ratio should not be specified in this range.

A description will now be given of embodiments of the invention in the following.

## Example 1

After the dermal layer of the hide of a young cow was cleaned and refined, it was cut to small pieces by a mincer. To the dermal layer thus minced was added a hydrochloric acid solution so as to be modified to a pH of 3. To the solution thus modified was added pepsin amounting to 2% by dry weight and the solution thus obtained was treated at 20°C for 48 hours. The solution was filtered and caustic soda was added to the filtered solution to modify the solution to a pH of 10 to deactivate pepsin. Thereafter the solution was modified to a pH of 7, and the settlings produced was collected from the solution and was washed well with water and again dissolved into a hydrochloric acid solution having a pH of 3. The solution was filtered and caustic soda was again added to the solution and modified to a pH of 7. The

settlings formed was collected from the modified solution and was washed with water, and then dissolved again in a hydrochloric acid solution having a pH of 3 to obtain a refined collagen of a concentration of 3.0 mg/ml.

Then, a refined human bone-forming factor obtained by the method described in the aforementioned literature was dissolved in normal hydrochloric acid of 0.01 to prepare a solution of a concentration of 1.0 mg/ml. Of the solution, 0.2 ml was poured into a test tube with 1.0 ml of the collagen solution added thereto and was well mixed. The mixture was freeze-dried, and then was sterilized with ethylene oxide gas to obtain a biomaterial.

The biomaterial was transplanted into the back muscle of a mouse and after three weeks the transplanted biomaterial was taken out to find that the material was replaced with 20 mg by wet weight of bone tissue.

#### Example 2

A square formed body made of hydroxylapatite and 5 mm long per side but lacking four corners and having a thickness of 2 mm and 40% porosity or a disc made of alumina ceramics was immersed in 1.2 ml of mixed solution of collagen and bone-forming factor described in Example 1 and was treated in vacuo to penetrate the mixed solution well into the ceramic formed body and then the body was subjected to freeze-drying and gas sterilization to obtain a biomaterial for transplantation. The biomaterial thus obtained was transplanted into the back muscle of a mouse and, after three weeks, the transplanted material was taken out to find the growth of a bone tissue on the surface of the second carrier. In this case, the result obtained when hydroxylapatite was used was substantially the same as that obtained from the use of alumina ceramics.

#### Example 3

Gelatin (viscosity: 44 mp; jelly strength: 253 Bloom; pH: 5.8; moisture: 11.0%) obtained by subjecting cow bone in the form of a material to normal lime treatment was dissolved in refined water to obtain a gelatin solution having a concentration of 50 mg/ml.

0.2 ml of hydrochloric acid solution of bone-forming factor described in Example 1 was mixed well with 1.0 ml of the gelatin solution, and was left to stand still in a refrigerator overnight to make the mixed solution form a gel. The material which gelated was immersed in 100 ml of phosphoric acid buffer solution (pH 7.2) containing 0.1% glutaric aldehyde at 5°C for 16 hours and was subjected to bridge treatment. Next, the material which gelated was taken out and was washed with refined water and thereafter subjected to freeze-drying and gas sterilization to obtain a biomaterial for transplantation.

The biomaterial was transplanted into the back muscle of a mouse to obtain substantially the same result as that in Example 1.

#### Example 4

The hydroxylapatite or alumina ceramic formed body used in Example 2 immersed in 1.2 ml of mixed solution of bone-forming factor and gelatine obtained in the same manner as in Example 3 and treated in vacuo to penetrate the mixed solution well into the ceramic body, and left to stand still in a refrigerator overnight to make the gelatin in ceramic body form a gel. The gelling material containing the ceramic formed body was treated with a phosphoric acid buffer solution containing glutaric aldehyde in the same manner as in Example 3, washed with water, and was subjected to freeze-drying and gas sterilization to obtain a biomaterial for transplanation. The biomaterial thus obtained produced the same result as that of Example 2.

As apparent from the embodiments of the invention described above, it has been demonstrated that in the artificial bone-forming biomaterial of the invention, biological function of the bone-forming factor is very low in species specificity. Judging from the embodiments, the invention is very effective in the clinical sphere of orthopedic surgery and oral surgery.

Incidentally, in the Examples 2 and 4, the embodiments in which the second carrier was impregnated with and carried by the mixed solution of the first carrier and the bone-forming factor were described by way of example, but in place of the impregnation and carrying of the mixed solution, the mixture may be attached merely to the second carrier. The attachment represents the state of the mixture being fixed to the surface portion of the second carrier, while the impregnation and carrying represents the state of the mixture being fixed not only to the surface but also to every corner of the interior of the second carrier, and accordingly, difference between the two is mere difference in the amount of bone formation.

Having described my invention as related to the embodiments, it is my intention that the invention be not limited by any of the details of description, unless otherwise specified, but rather be construed broadly within its spirit and scope as set out in the accompanying claims.

#### CLAIMS

1. An artificial bone-forming biomaterial comprising: a mixture of bone-forming factor and a first carrier for said factor, said carrier being selected from collagen and derivatives thereof and denatured substances.

# POOR QUALITY

5

GB 2 164 042 A

5

2. An artificial bone-forming biomaterial comprising: a mixture of bone-forming factor and a first carrier for said factor, said carrier being selected from collagen, derivatives thereof and denatured substances, and a second carrier for said mixture, said carrier being a formed body of ceramic, metallic or synthetic resinous material wherein said mixture is attached to or impregnated into and carried by said second carrier. 5
3. An artificial bone-forming biomaterial according to Claim 1 or 2 wherein said bone-forming factor is a bone-forming extracted from Dunn osteosarcoma or a human bone-forming factor extracted from a human osteosarcoma. 5
4. An artificial bone-forming biomaterial according to Claim 1 or 2 wherein said mixture of first carrier and bone-forming factor consists of mixed solutions of the respective liquids of said carrier and bone-forming factor. 10
5. An artificial bone-forming factor according to Claim 4 wherein said first carrier is a hydrochloric acid solution of collagen or gelatin and said bone-forming factor is a hydrochloric acid solution of human bone-forming factor. 10
6. An artificial bone-forming biomaterial according to Claim 1 wherein said mixture of first carrier and bone-forming factor is a freeze-dried mixture of a mixed liquid of the respective liquids of said first carrier and said factor. 15
7. An artificial bone-forming biomaterial according to Claim 1 wherein said mixture of first carrier and bone-forming factor is a gelled substance of a mixed liquid of the respective liquids of said carrier and said factor. 20
8. An artificial bone-forming biomaterial according to Claim 2 wherein said second carrier is a formed body of an hydroxylapatite or alumina ceramic. 20
9. An artificial bone-forming biomaterial according to Claim 8 wherein said second carrier is impregnated with said mixture of first carrier and bone-forming factor. 20
10. An artificial bone-forming biomaterial according to Claim 9 wherein said mixture liquid is formed into a gel and freeze-dried. 25
11. A method of making an artificial bone-forming biomaterial, said method comprising mixing a liquid of a first carrier for a bone-forming factor with a liquid of said bone-forming factor, said first carrier being selected from collagen, derivatives thereof, and denatured substances derived therefrom. 30
12. A method of making an artificial bone-forming biomaterial, said method comprising causing a mixed liquid of a first carrier for a bone-forming factor and a liquid of said bone-forming factor to be attached to or impregnated into and carried by a second carrier of formed body of any one of ceramics, metal and synthetic resin, said first carrier being selected from collagen, derivatives thereof, and denatured substances derived therefrom. 30
13. A method of making an artificial bone-forming biomaterial according to Claim 12 wherein said first carrier is a hydrochloric acid solution of collagen or an aqueous solution of gelatin and said bone-forming factor solution of human bone-forming factor separated and refined from human osteosarcoma. 35
14. A method of making an artificial bone-forming biomaterial according to Claim 12 or 13 wherein said second carrier is hydroxylapatite or alumina ceramics. 35
15. A method of making an artificial bone-forming biomaterial according to Claim 11 or 13, said method further comprising the step of freeze-drying a mixed liquid of said first carrier and bone-forming factor. 40
16. A method of making an artificial bone-forming biomaterial according to Claim 11 or 13, said method further comprising the step of forming a gel of said mixed liquid of first carrier and bone-forming factor. 45
17. A method of making an artificial bone-forming biomaterial according to Claim 12 or 13, said method further comprising the step of forming a gel of and then freeze-drying a mixed liquid of first carrier impregnated with and carried by said second support. 45
18. A method of making an artificial bone-forming biomaterial according to any one of Claims 15 to 17, said method further comprising the step of sterilization by means of ethylene oxide gas. 50